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RESEARCH ARTICLE

Laser Acupuncture Improves Memory Impairment in an Animal Model of Alzheimer's Disease



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Abstract

The burden of Alzheimer's disease is continually rising globally, especially in the Asia-Pacific region. Unfortunately, the efficacy of the therapeutic strategy is still very limited. Because the effect of acupuncture at HT7 can improve learning and memory, the beneficial effect of laser acupuncture, a noninvasive form of acupuncture, at HT7 on memory improvement in patients with Alzheimer's disease has been a focus of research. To elucidate this issue, we used AF64A, a cholinotoxin, to induce memory impairment in male Wistar rats, which weighed 180–220 g. Then, the animals were treated with laser acupuncture either at HT7 or at a sham acupoint once daily for 10 minutes for a period of 14 days. Spatial memory assessments were performed at 1, 7, and 14 days after AF64A administration and at the end of the experiment, and the changes in the malondialdehyde (MDA) level and in the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and acetylcholinesterase (AChE) activities in the hippocampus were recorded. The results showed that laser acupuncture significantly suppressed AChE activity in the hippocampus. Although laser acupuncture enhanced SOD and CAT activities, no reduction in MDA level in this area was observed. Therefore, laser

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acupuncture at HT7 is a potential strategy to attenuate memory impairment in patients with Alzheimer's disease. However, further research, especially on the toxicity of laser acupuncture following repetitive exposure, is essential.

1. Introduction

To date, the burden of Alzheimer's disease has been continually increasing globally, particularly in developing countries, many of which lie in the Asia-Pacific region [1]. It is the most common age-related neurodegenerative disorder and dementia and can greatly impair the quality of life of patients as well as their families and caregivers [2]. This condition cannot be completely cured; most treatments can only slow down the progression of the disease. Most medications used for treating Alzheimer's disease target cholinergic improvement, and they usually have adverse effects [3]. Therefore, alternative treatments are required.

Acupuncture is one of the popular complementary alternative medicine methods used for treating dementia, and it can effectively improve intelligence [4]. Several lines of evidence also clearly demonstrate the cognitive-enhancing effect of needle acupuncture at shenmen or HT7 [5]. However, because needle acupuncture is an invasive technique, patients who do not like invasive techniques may not want to endure the tingling sensation induced by needle piecing; thus, laser acupuncture (LA) was developed to avoid this problem. LA is the application of photobiostimulation, instead of needle stimulation, at an acupoint. Because LA is a noninfectious and easy to use technique and because it can avoid the pain and psychological fear associated with needle acupuncture, it can be used to study the potential to improve memory in an animal model of Alzheimer's disease.

2. Materials and methods

2.1. Animals

Young (8-week-old) adult male Wistar rats were used as experimental animals. They were obtained from the National Animal Center, Salaya. On the 1st day of the experiment, the animals weighed 180–220 g. They were housed six per cage, maintained in a 10:14 light/dark cycle, and given *ad libitum* access to food and water. The experiments were performed to minimize animal suffering, and the experimental protocols were approved by the Institutional Animal Care and Use Committee, Khon Kaen University, Thailand. All treatments in this study were performed once daily between 8:00 A.M. and 5:00 A.M.

2.2. Chemicals and surgical procedures

Ethylcholine aziridinium (AF64A) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The animals were anesthetized by intraperitoneal injection of sodium barbital (Jagsonpal Pharmaceuticals LTD, Haryana, India) at a dose of 60 mg/kg body weight. Next, AF64A (2 nmol/2 μ L) was bilaterally infused via an intracerebroventricular route

with a 30-gauge needle via burr holes that had been drilled through the skull into both the right and the left lateral ventricles according to the following stereotaxic coordinates: posterior 0.8 mm, lateral ± 1.5 mm, and ventral (from dura) 3.6 mm. The rate of infusion was 1.0 μ L/min. The needle was left in place for 5 minutes after infusion and then slowly withdrawn.

2.3. AF64A administration

AF64A was prepared as previously described [6]. Briefly, an aqueous solution of acetylthylcholine mustard HCl (Sigma) was adjusted to pH 11.3 with NaOH. After the solution had been stirred for 30 minutes at room temperature, the pH was lowered to 7.4 with the gradual addition of HCl and was again stirred for 60 minutes. The amount of AF64A was then adjusted to 2 nmol/2 μ L. Distilled water was processed in the same manner as the preparation of AF64A and was designated as artificial cerebrospinal fluid (ACSF).

2.4. Experimental protocol

All rats were randomly assigned to six groups of six animals each as follows. In Group I (control group), the rats received no treatment. In Group II (vehicle group), the rats were administered ACSF bilaterally via an intracerebroventricular route. In Group III (vehicle + LA group), the rats were administered ACSF via an intracerebroventricular route and were subjected to LA treatment bilaterally at HT7. In Group IV (AF64A group), the rats received intracerebroventricular administration of AF64A, a cholinotoxin. In Group V (AF64A + sham LA group), the rats received intracerebroventricular administration of AF64A and LA treatment at a nonacupoint. In Group VI (AF64A + LA group), the rats received intracerebroventricular administration of AF64A and LA treatment at the HT7 acupoint.

The rats were treated with LA once daily for 14 days after the administration of AF64A. Then, they were assessed for spatial memory by using the Morris water maze test at days 1, 7, and 14 after AF64A injection. At the end of the experiment, they were sacrificed, and their brains were isolated to identify oxidative damage markers, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). In addition, the activity of acetylcholinesterase (AChE) in the hippocampus, a crucial area for learning and memory, was determined.

2.5. LA treatment protocol

Fifteen minutes before LA treatments, all rats were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneal injection) to minimize stress. LA treatment was performed once daily for 14 days. The rats were treated with a laser instrument that operated with a continuous

violet or blue laser beam (Xinland International Limited, Hongguang Rd, Lianhu District, Xi'an, Shaanxi, China) at a wavelength of 405 nm, an output power of 100 mW, and a spot diameter of 500 μm for 10 minutes [7,8]. LA was applied in the rats either at the HT7 point (the transverse crease of the wrist of the forepaw, radial to the tendon of the muscle flexor carpi ulnaris) or at a point 2–4 mm lateral to the HT7 acupoint [9].

2.6. Determination of cognitive function

Cognitive function was evaluated using the Morris water maze test. The water maze consisted of a metal pool [170 cm (diameter) \times 58 cm (height)] filled with tap water (25 $^{\circ}\text{C}$, 40 cm deep). The pool was divided into four quadrants (northeast, southeast, southwest, and northwest). The water surface was covered with nontoxic milk. A removable platform was immersed below the water's surface at the center of one quadrant. For each animal, the location of the invisible platform was placed at the center of one quadrant and was kept at that location throughout training. The time that each animal spent to find and climb onto the hidden platform was recorded as the escape latency. In order to determine the capability of the animals to retrieve and retain information, the platform was removed 24 hours later, and the rats were released into the quadrant diagonally opposite the quadrant that contained the platform. The time that each animal spent in the region that had previously contained the platform was recorded as the retention time [10].

2.7. Determination of oxidative stress markers and AChE activity

Rats were perfused with a cold saline solution to remove the blood from the brain tissue; then, the hippocampi were rapidly removed and stored at 80 $^{\circ}\text{C}$ until used. To determine the oxidative stress markers and the AChE activity, we prepared the brains as homogenates, and we determined the MDA level using the thiobarbituric acid reaction whereas we determined the GSH-Px, CAT, and SOD activities with a spectrophotometric method.

2.8. Statistical analysis

Data were expressed as mean \pm standard error of the mean (SEM) and were analyzed statistically using the one-way ANOVA test, followed by the *post hoc* (least significant difference) test. The results were considered statistically significant at $p < 0.05$.

3. Results

3.1. Cognitive-enhancing effect of LA

The effects of LA on cognitive function are shown in Figs. 1 and 2. ACSF did not produce any significant changes in the escape latency and the retention time, whereas rats treated with either AF64A or AF64A + LA at a sham acupoint showed an elevated escape latency, but a decreased retention time ($p < 0.001$ for all compared to the ACSF group), throughout the study period. The administration of LA reversed the elevation of escape latency at 7 and 14 days after AF64A administration ($p < 0.05$ and 0.001 , respectively, compared to the AF64A-treated group), whereas no significant change was observed in rats treated with AF64A plus LA at a sham acupoint. In addition, LA at a sham acupoint did not produce any significant change in rats that received ACSF.

Fig. 2 shows that rats that received AF64A had reduced retention times at 7 days and 14 days after AF64A administration ($p < 0.001$ for all compared to the ACSF group). LA at the HT7 acupoint was found to be able to reverse the decreased retention time induced by AF64A ($p < 0.01$ and $p < 0.001$, respectively, compared to the AF64A-treated group). Again, no significant change in retention time was observed in rats that received ACSF plus LA at a sham acupoint.

3.2. Effect of LA on oxidative stress markers and AChE enzyme activity

Figs. 3–6 show the effects of LA on the oxidative stress markers, including the MDA level and the activities of SOD,

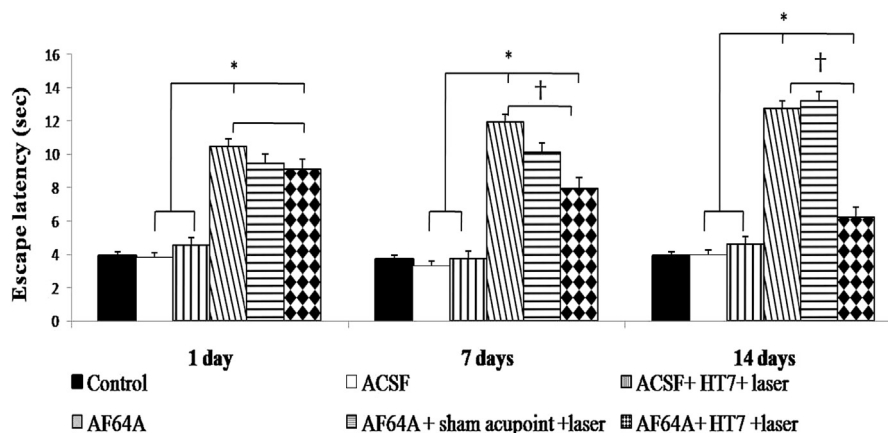


Figure 1 Effect of laser acupuncture on escape latency using the Morris water maze test in rats with Alzheimer's disease. Values given are the mean \pm SEM ($n = 6$). * $p < 0.001$ compared with the ACSF group. † $p < 0.001$ compared with the AF64A group. ACSF = artificial cerebrospinal fluid; SEM = standard error of the mean.

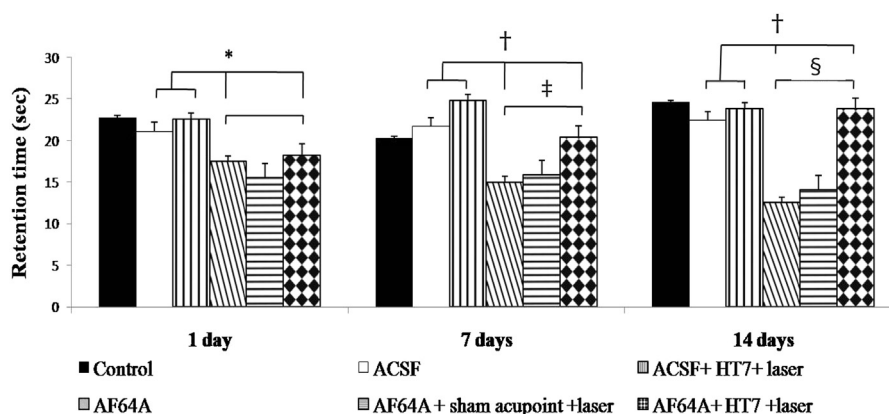


Figure 2 Effect of laser acupuncture on retention time using the Morris water maze test in rats with Alzheimer's disease. Values given are the mean \pm SEM ($n = 6$). * $p < 0.01$, † $p < 0.001$ compared with the ACSF group. ‡ $p < 0.01$, § $p < 0.001$ compared with the AF64A group. ACSF = artificial cerebrospinal fluid; SEM = standard error of the mean.

CAT, and GSH-Px in the hippocampus. ACSF did not produce any significant changes in either the MDA level or the SOD, CAT, and GSH-Px activities. Rats treated with AF64A had significantly enhanced MDA levels, but decreased CAT activities ($p < 0.01$ and $p < 0.001$, respectively, compared to the ACSF group). Interestingly, rats that underwent LA at

the HT7 acupoint showed enhanced CAT and SOD activities, but no changes in GSH-Px activity and MDA level were observed.

The effect of LA on AChE activity in the hippocampus is shown in Fig. 7. ACSF produced no change in AChE activity. Rats treated with either AF64A or AF64A plus LA at a sham acupoint showed elevated AChE activity in the hippocampus. However, this elevation was attenuated by LA at the HT7 acupoint.

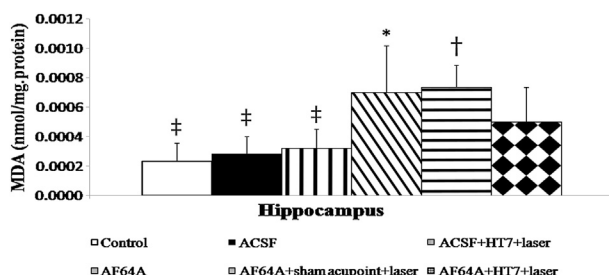


Figure 3 Effect of laser acupuncture on the level of MDA, a product of lipid peroxidation, in the hippocampus. Values given are the mean \pm SEM ($n = 6$). * $p < 0.01$, † $p < 0.001$ compared with the ACSF group. ‡ $p < 0.001$ compared with the AF64A group. ACSF = artificial cerebrospinal fluid; MDA = malondialdehyde; SEM = standard error of the mean.

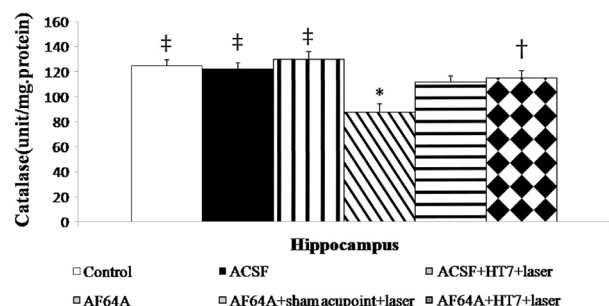


Figure 4 Effect of laser acupuncture on the activity of CAT in the hippocampus. Values given are the mean \pm SEM ($n = 6$). * $p < 0.001$ compared with the ACSF group. † $p < 0.01$, ‡ $p < 0.001$ compared with the AF64A group. ACSF = artificial cerebrospinal fluid; CAT = catalase; SEM = standard error of the mean.

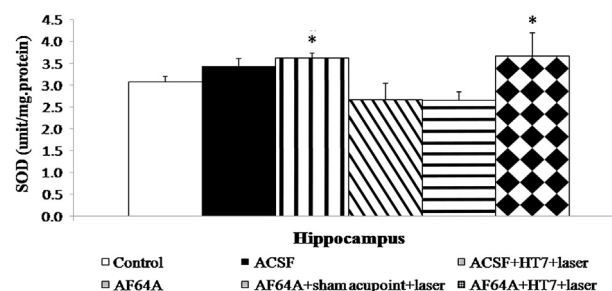


Figure 5 Effect of laser acupuncture on the activity of SOD in the hippocampus. Values given are the mean \pm SEM ($n = 6$). * $p < 0.05$ compared with the AF64A group. SEM = standard error of the mean; SOD = superoxide dismutase.

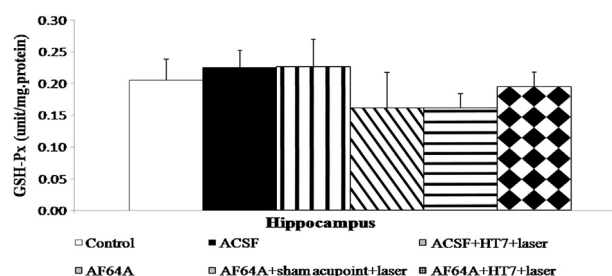


Figure 6 Effect of laser acupuncture on the activity of GSH-Px in the hippocampus. Values given are the mean \pm SEM ($n = 6$). GSH-Px = glutathione peroxidase; SEM = standard error of the mean.

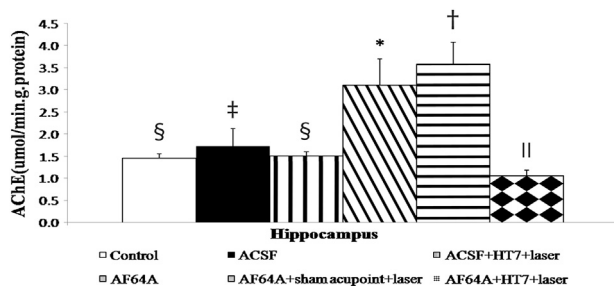


Figure 7 Effect of laser acupuncture on the activity of AChE in the hippocampus. Values given are the mean \pm SEM ($n = 6$). * $p < 0.05$, † $p < 0.01$ compared with the ACSF group. ‡ $p < 0.05$, § $p < 0.01$, || $p < 0.001$ compared with the AF64A group. AChE = acetylcholinesterase; ACSF = artificial cerebrospinal fluid; SEM = standard error of the mean.

4. Discussion

This study demonstrates the cognitive-enhancing effect of LA and its positive modulation effect on oxidative stress and cholinergic function.

In this study, a blue or violet laser beam at a wavelength of 405 nm was applied at the HT7 acupoint. Because the laser beam used in this study was a low-energy beam, this treatment is also referred to as low-level laser therapy (LLLT). LLLT is believed to produce photochemical, rather than thermal, effects because low irradiation levels are used and because no appreciable temperature rise takes place [11]. LLLT may decrease the oxidative stress in oxidatively stressed neurons [12]. However, rats that had undergone LA at sham acupoints, both in the ACSF and the AF64A-treated groups, showed no changes in MDA levels in their hippocampus. A possible explanation for the discrepancy between our study and the aforementioned study might be the differences in the selected brain areas and the types of exposure. This study was an *in vivo* study, whereas the previous study was an *in vitro* study.

LA at HT7 significantly enhanced the CAT and the SOD activities without significantly changing the MDA level in the hippocampus. These data suggest that the restoration of oxidative stress balance might not play an important role in the cognitive-enhancing effect of LA. In contrast to the MDA change, rats subjected to LA showed suppressed elevations of AChE activity, which in turn indirectly reflected the elevation of ACh in the hippocampus. Because ACh plays an important role in learning and memory and because the AChE inhibitor can also reduce memory impairment in Alzheimer's disease, we suggest that LA at HT7 may improve cholinergic function in the hippocampus, which in turn gives rise to enhanced spatial memory capacity in an animal model of Alzheimer's disease, as observed in this study.

In conclusion, this study is the first study to demonstrate the positive modulation effect of LA at HT7 on the cholinergic function, which in turn leads to reduced cognitive impairment in an animal model of Alzheimer's disease.

Therefore, LA at HT7 is a potential noninvasive strategy to attenuate memory impairment. Because LA is very new and little information is available, detailed research, especially on the toxicity of repetitive exposure, is essential.

Disclosure statement

The author affirms there are no conflicts of interest and the author has no financial interest related to the material of this manuscript.

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